# STUDIES ON THE INHIBITION OF ANAPHYLACTIC HISTAMINE RELEASE FROM THE GUINEA-PIG LUNG BY β-ADRENOCEPTOR STIMULANTS

by

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III The β-adrenoceptors of the lung mediating inhibition of antigen-induced histamine release.
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IV Inhibition of antigen-induced tone in isolated trachea.
European J. Pharmacol. 1975, 31, 374-376.

V Pharmacological properties of 1-(3'5'-diisobutyryloxyphenyl)-2-(t-butylamino)-ethanol-hydrochloride (KWD 2058), a new sympathomimetic bronchodilator.
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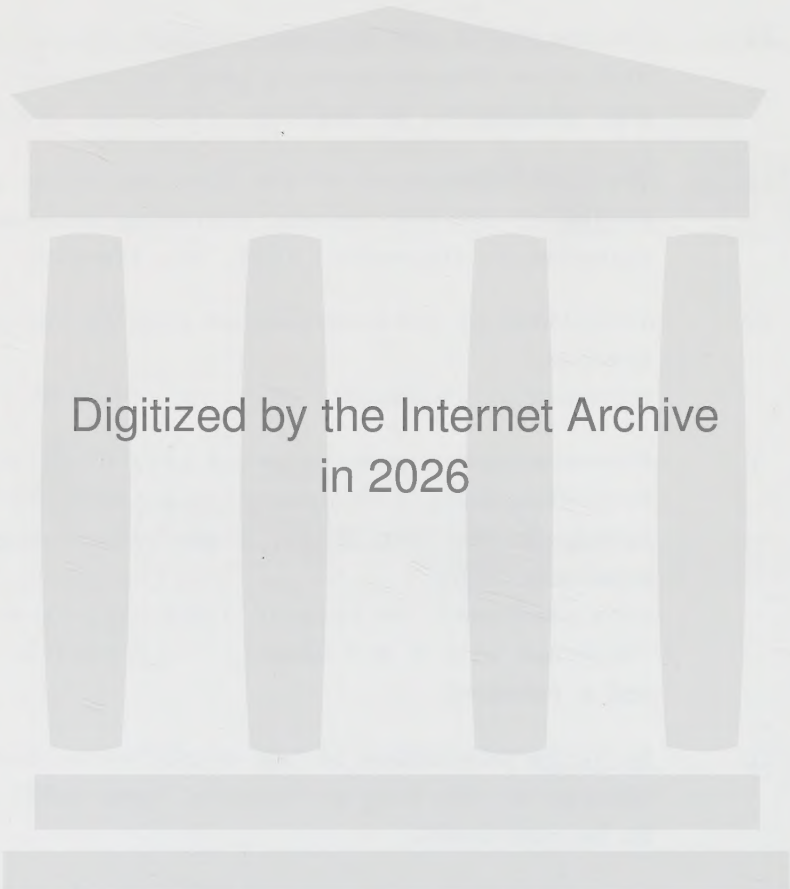
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ABBREVIATIONS

| | |
|---|---|
| cAMP | cyclic adenosine 3'5'-monophosphate |
| COMT | catechol-O-methyltransferase |
| EDTA | ethylendiamine tetraacetic acid |
| FFA | free fatty acids |
| 5HT | 5-hydroxytryptamine |
| Ig | immunoglobulin |
| IPR | isoprenaline sulphate |
| ITP | 1-isopropylamino-3-(2-thiazoloxy)-2-propanol HCl |
| MAO | monoamine oxidase |
| OPR | orciprenaline sulphate |
| PG | prostaglandin |
| RCS | rabbit aorta contracting substance |
| SRS-A | slow reacting substance of anaphylaxis |
| TRB | terbutaline sulphate |

# I INTRODUCTION

Sympathomimetic β-adrenoceptor stimulating agents have proved to be of great value in the treatment of obstructive lung diseases by relaxing the bronchial smooth muscle. Besides the main effect, unwanted side effects have been described, i.e. increase in heart rate, palpitations, and development of tremor.

In 1936, Schild discovered the anti-anaphylactic effect of adrenaline revealed as inhibition of antigen-induced release of histamine from sensitized guinea-pig lung. Assem and Schild in 1969 emphasized the importance of this additional beneficial effect of β-adrenoceptor stimulants by the discovery that catecholamines were effective as anti-anaphylactic agents in very low concentrations. They therefore proposed that 'the clinical effectiveness of isoprenaline and adrenaline in allergic bronchial asthma depends on the ability of these compounds both to inhibit the anaphylactic mechanism and to relax the bronchial smooth muscle'.

The present thesis deals with the anti-anaphylactic effect of β-adrenoceptor stimulants and pharmacological characterization of the adrenoceptor which mediates this effect in the lung. The traditional immunological model of antigen-induced airway obstruction, the guinea-pig, has been used throughout the study. Pharmacological tools for the study are mainly β-adrenoceptor stimulants with different profiles of action.

## 1. Immediate hypersensitivity reaction in the lung

The experimentally induced type I immediate allergic reaction (Gell and Coombs 1968), designated as anaphylaxis, has been extensively reviewed by Mongar and Schild (1962). It can be described as a sequence of events by the interaction of specific antigen with tissue

sensitizing antibody leading to the activation of cellular enzymes. This activation will bring about the appearance of pharmacologically active agents (i.e. histamine, SRS-A, and PG:s) which will cause bronchoconstriction, capillary leakage, and other pathophysiological manifestations.

Most species contain two homologous antibodies capable of participating in this type of reaction (Bloch 1967): one is the IgE type, which is an electrophoretically fast, heat labile group of antibodies found in serum in trace amounts. They can be detected after an intracutaneous administration to intact rats several weeks later. The other is the IgG type, which is electrophoretically slow, heat stable, and present in mg amounts in serum. It persists at an intracutaneous administration for some hours up to 2 days. In the guinea-pig, $IgG_1$ antibodies (Benacerraf *et al*. 1963) and antibodies with physicochemical characteristics similar to human IgE antibodies (Catty 1969, Dobson *et al*. 1971) are known to take part in anaphylaxis.

The pharmacological mediators, currently considered to be involved in immediate-type hypersensitivity reactions of the guinea-pig lung, have been extensively reviewed (Collier and James 1970, Orange 1973). They can be regarded as two groups: (1) small, chemically well-defined molecules: histamine, 5HT, acetylcholine; and (2) larger more or less well defined compounds: SRS-A, PG:s, kinins, and rabbit aorta contracting substance (RCS). Of these agents histamine, 5HT, PG:s, and acetylcholine are to be found preformed in the lung; the other compounds appear during anaphylaxis.

Similar to histamine and 5HT, SRS-A is released as a direct result of the antigen-antibody reaction in the lung (Kellaway and Trethewie 1940, Brocklehurst 1960, Chakravarty and Uvnäs 1960), whereas PG:s are probably formed as a result of the constrictive role of released

histamine and SRS-A on smooth muscle (Palmer et al. 1973). Bradykinin is produced in anaphylaxis (Brocklehurst and Lahiri 1962), secondary to immunological tissue injury (Kaplan and Austen 1970). RCS is less well established, and is thought to be an active compound in the PG system (Piper and Vane 1969, Gryglewski and Vane 1971). Acetylcholine, finally, is also released secondary to anaphylaxis. It is involved in the reflexogenous bronchoconstriction caused by the action of histamine on 'irritant receptors' in the bronchial epithelium (Mills and Widdicombe 1970).

Of the released mediators, histamine and SRS-A are ascribed the most important bronchoconstrictive roles in guinea-pig anaphylaxis (Collier and James 1967, Drazen et al. 1973, Strandberg and Hedqvist 1975). $PGF_{2\alpha}$ contributes to a less extent to the airway resistance in the guinea-pig (Berry and Collier 1964). This is also valid for 5HT and bradykinin (Collier and James 1967). Whereas the increase in airway resistance is partly caused by vagal reflex, the appearance of rapid shallow breathing is almost entirely reflexogenous (Mills and Widdicombe 1970).

## 2. Sympathomimetic agents as tools for classification of adrenoceptors

The drug receptor concept was applied by Ahlquist (1948) to the adrenergic stimulants noradrenaline and adrenaline. The receptors involved were classified by him in two groups, α- and β-adrenoceptors. The base for this differentiation was rank order of potency, in various organs, of five sympathomimetic amines structurally related to noradrenaline. He found striking differences in the profile of action of these compounds; for instance, adrenaline and noradrenaline were most potent in causing excitatory (α) responses,i.e. contraction of uterus, blood vessels, and ves. seminalis, whereas IPR was most

potent in causing inhibitory (β) responses, i.e. relaxation of the muscles in the bronchi, blood vessels, and uterus. Exceptions were the α-adrenoceptors of the intestine, which were inhibitory (intestinal relaxation) and the β-adrenoceptors of the heart, which were excitatory (heart stimulation). Further work along this line was done by Lands and co-workers (Lands and Brown 1964, Lands et al. 1966, 1967). They studied the relative potencies of series of β-adrenoceptor stimulants in producing responses both in isolated organs and in animals. They made statistical comparisons of the potency series obtained for different responses, and they proposed that the β-adrenoceptor population was heterogenous. Their results caused them to postulate the existence of two types of β-adrenoceptors, termed $\beta_1$ and $\beta_2$. They found, for instance, good correlation between cardiac stimulation and lipolysis, on one hand ($\beta_1$-effects), and bronchodilatation and vasodilation, on the other ($\beta_2$-effects). Bergman et al. (1969) reported on a separation of the effect on tracheal and heart muscle with two series of 3-5-dihydroxy N-substituted compounds. The conclusions by Lands and co-workers, based on effects obtained in various mammalian species, were strengthened by Persson and Johnson (1970), who studied the effect on heart and bronchial muscle preparations taken from a guinea-pig under identical experimental conditions.

### 3. Interference of sympathomimetics with the anaphylactic mediator release in the lung

In 1936, Schild reported on the inhibitory effect of high concentrations of adrenaline on ovalbumin-induced histamine release from the isolated and perfused guinea-pig lung. Giotti et al. (1966) studied anaphylaxis in guinea-pig hearts. They found that reserpine as well as the β-blocking agents dichloroisoprenaline and pronethalol reduced the histamine release and discussed a modulating role of catecholamines in cardiac anaphylaxis.

Lichtenstein and Margolis (1968) found that catecholamines, and also methylxanthines, reduced the histamine release from human leucocytes. They suggested that the drugs inhibited anaphylactic histamine release by increasing the intracellular level of cAMP. Assem and Schild (1969) followed up the latter's findings in perfused lung by investigating the same effect in chopped human lung. In this system adrenaline and IPR were effective as inhibitors in very low concentrations, $10^{-11}$ - $10^{-10}$ M. Noradrenaline was less potent, indicating that the inhibition of histamine release is mediated by β-adrenoceptors. The inhibitory effect was also shown with the chopped guinea-pig lung (Assem *et al*. 1970) and primate lung (Ishizaka *et al*. 1971). The anaphylactic SRS-A (Liebig *et al*. 1974) and PG release (Mathé and Levine 1973) was counteracted by catecholamines in the guinea-pig lung.

## II AIMS OF THE PRESENT STUDY

The aims of the present study are summarized thus:

1/ Characterization of the β-adrenoceptors of the guinea-pig lung mediating inhibition of anaphylactic histamine release. Tools for this classification are β-adrenoceptor stimulants with different profiles of action on guinea-pig trachea and heart. The rank order of potency will indicate whether the anti-anaphylactic β-adrenoceptors are of the same quality as those responsible for trachea relaxation, or those responsible for increase in heart rate and force, or whether they should be considered a special class of β-adrenoceptors.

2/ The protecting effect of β-adrenoceptor stimulants against anaphylactic bronchospasm is thought to be exerted by action on two levels in the chain of events: inhibition of anaphylactic mediator release; relaxation of bronchial smooth muscle (Assem and Schild 1969). Based on this, a β-adrenoceptor stimulant would be more effective against the development of anaphylactic bronchospasm than against the same amount of bronchospasm elicited by the addition of mediators. The anti-anaphylactic effect of terbutaline is specially studied in relation to relaxation of tracheal smooth muscle and protection against different kinds of respiratory distress in guinea-pigs.

3/ Pharmacological characterization of the diisobutyric acid ester of terbutaline, ibuterol (KWD 2058), and its effect on anaphylactic histamine release.

## III CHARACTERIZATION OF THE β-ADRENOCEPTOR STIMULANTS

Figure 1 surveys the chemical configuration and relative potencies on guinea-pig trachea and heart.

### Isoprenaline (IPR)

The most potent of the agonists used in this study is IPR, described by Konzett (1941). According to the classification of Lands, it is nonselective, i.e. it stimulates the heart and relaxes the guinea-pig trachea in the same concentrations (Persson and Johnson 1970). O´Donnell and Wanstall (1974) found that IPR was slightly more potent on the auricle than on the trachea.

### Orciprenaline (OPR)

Orciprenaline (Engelhardt et al. 1961) has the same nonselective profile as IPR, although it is less potent (Engelhardt and Traunecker 1972).

### ITP

ITP is a myocardial stimulant without vasodepressive effect, as revealed in the dog (Roszowski et al. 1972). Lockwood and Lum (1974) found that ITP increased the plasma levels of FFA in the cat without affecting the levels of blood glucose or blood lactate. It is a partial agonist on the guinea-pig heart with a very weak relaxing effect on the trachea (III). Thus ITP shows the same pattern of stimulation as a $\beta_1$-selective stimulating agent. It blocks the IPR effects in the two mentioned preparations, thereby fulfilling the criteria of a partial agonist according to Ariens et al. (1964).

### Terbutaline (TRB)

TRB is predominantly a $\beta_2$-selective stimulating agent (Persson and Olsson 1970). It relaxed the isolated trachea in a concentration range where it showed no auricle stimulation (Persson and Johnson 1970). TRB is a full agonist on the trachea, whereas it shows weak stimulating effects on the isolated guinea-pig heart (III).

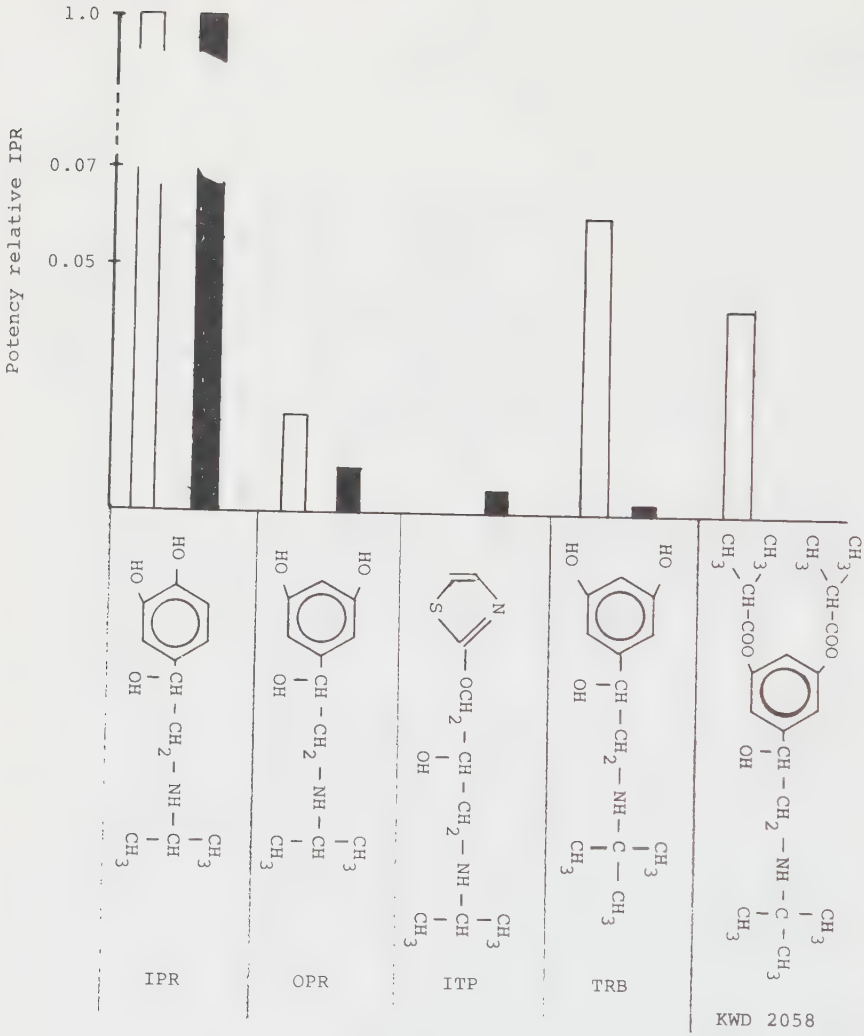


Fig 1

The relative potency of IPR, OPR, ITP, TRB and KWD 2058 on the isolated guinea-pig trachea (relaxation), white bars, and on the isolated and perfused heart (force of contraction), black bars.

Ibuterol (KWD 2058)

Ibuterol is the diisobutyric acid ester of TRB (Fig 1).

Paper V lists some pharmacological effects of this compound. Ibuterol relaxed the isolated tracheal and bronchial muscles of cats and guinea-pigs to the same extent as TRB. The cardiac effects, although less pronounced, were qualitatively similar to TRB. In vivo, ibuterol lowered the arterial mean pressure and heart rate of cats and dogs. In anaesthetized cats, it inhibited the bronchoconstricting effect of histamine in doses where the effect on the heart was very small. It is concluded that the profile of action of ibuterol is that of a selectively-acting bronchodilating agent.

In sera from homo, dog, and cat, ibuterol is rapidly hydrolyzed to TRB and isobutyric acid (Kristoffersson et al. 1974). In isolated cat bronchial preparations, pretreatment with the esterase inhibitor eserine made the preparation almost insensitive to ibuterol in concentrations that relaxed control preparations. TRB, however, relaxed the eserine-treated preparation in the same concentrations as in the controls. Diisobutyric acid had no effect on the trachea. The relaxing effect of ibuterol was blocked with propranolol. These findings suggest that ibuterol undergoes hydrolyzation to TRB before it is effective as a β-stimulating agent.

The most obvious differences between ibuterol and TRB were: the better oral availability of ibuterol, found in conscious guinea-pigs and in toxicity studies in mice. There was also a tendency to less cardiovascular effects of ibuterol than of TRB at the same bronchodilating effect. As ibuterol is ineffective per se and effective after hydrolyzation to TRB, it can be regarded as a predrug for TRB.

None of the above-mentioned compounds, except IPR, showed α-adrenoceptor stimulating effect when tested in

concentrations ≤ 25 µg/ml on the rabbit aortic strip (III, V). IPR caused α-stimulation in the concentration 3 µg/ml. However, the concentrations used in the experiments on lung anaphylaxis were far below those causing α-stimulation.

The agonists used did not restore the stimulating effect of tyramine in the reserpinized rabbit heart (Andén et al. 1964, III), which is taken as indirect evidence that the 4 agonists used in the rank order experiments (III) are not taken up into nerves.

Catechol-O-methyltransferase (COMT) is an enzyme of importance for inactivation of IPR (Axelrod and Tomchick 1958). TRB, OPR, and ITP lack OH-group in the 4-position of the benzene ring; therefore they are not attacked by COMT (Persson and Persson 1972). Compounds with isopropyl- or t-butyl substituents at the nitrogen atom are not attacked by monoamine oxidase (MAO) (Blaschko 1952, Persson and Persson 1972). Thus the compounds used are not substrates for MAO.

## IV METHODS FOR STUDIES ON ANAPHYLAXIS

### 1. Chopped lung

The experimental model used in the present study was essentially that introduced by Mongar and Schild (1953) and later applied by Assem and Schild (1971 a) on studies of the inhibition of sympathomimetic amines on histamine release from guinea-pig lung. Guinea-pigs were sensitized by a single intraperitoneal injection of ovalbumin in Freund´s complete adjuvant. The adjuvant was used in order to improve the sensitization (Freund and Mc Dermott 1942, Hicks and Skeldon 1972). Two to four weeks later, the animals were stunned with a blow on the head and bled to death. The lungs from 2-4 animals were excised, pooled, and thoroughly minced. The tissue was incubated in Krebs solution at 37°C. The antigen was added after 5 minutes´ preincubation, and the incubation continued for 15 minutes. The histamine content of the incubation fluids and the boiled tissue extracts was determined by bioassay on the atropinized and β-blocked guinea-pig ileum. The rapid contractions blockable with brompheniramine were compared with those of known amounts of histamine hydrochloride.

There was an increase of histamine in the incubation fluid and a concomitant decrease of histamine in the challenged tissue that significantly differed from spontaneous loss and could be referred to the action of ovalbumin. The histamine release was found to depend on the concentration of ovalbumin and the time of incubation with antigen (I, II). Generally, ovalbumin, 1 mg/ml, caused histamine release which varied from 15 to 30 % of that extractable from the lung tissue. These values agree with those of Assem and Schild (1971 a). Incubation times exceeding 15 minutes did not increase the histamine content of the incubation fluid (I). Chakravarty (1960) reported that histamine appeared in the incubation fluid within a few seconds and showed a peak between 8 and 16 minutes. Austen and Brocklehurst (1961) found that 50 % of the

histamine release from chopped guinea-pig lung induced with ovalbumin was obtained in less than 1 minute, almost all being released in 5 minutes. The same technique applied to human lung showed that maximum release of histamine was obtained within 5 minutes (Orange et al. 1971).

2. Guinea-pig trachea

Spirally cut tracheal preparations from sensitized guinea-pigs were mounted in water baths with Krebs solution at 37°C. The isometric tension was registered. Increased tension was produced with ovalbumin. By intermittent washing of the preparation during about 1 h, the response (25 - 35 % of maximum contraction) could be reproduced 4 times (IV). The inhibitory effect of low concentration of TRB on the tone was studied either when added 5 minutes before ovalbumin or when added at the peak effect of the contracting agent. The TRB effect was also studied when ovalbumin was replaced by histamine, acetylcholine, or $PGF_{2\alpha}$.

Although there is direct evidence of the involvement of mediators in the anaphylactic reaction of the guinea-pig ileum (Cirstera and Suhaciu 1968, Kurihara and Shibata 1972) there is only indirect evidence of mediators being involved in the anaphylactic contraction of the isolated trachea.

Mepyramine, when added in a concentration that completely blocked the mechanical response of histamine, reduced the response of treatment with ovalbumin to 59 % of control (Joiner et al. 1974). The residual contractility was slower in onset, which argues for SRS-A as a possible candidate for this non-histamine response.

Dale (1965) found that the guinea-pig ileum frequently recovered the sensitivity to the previously-given amount of antigen. This "resensitization" has been carefully

studied by Dale and Okpako (1969). They explained the recovery by dissociation of antigen-antibody complex, thereby making antibody sites available again for the antigen. The repeated anaphylactic response in the trachea experiments presented here (IV) might to some extent depend on "resensitization", as there was a need for several washings before complete recovery of response. But the low dose of antigen used might also have left antibody sites free for participation in the following challenge. Lewis et al. (1974), who studied the formation and release of SRS-A from human lung tissue, found that low doses of antigen stimulated the formation of SRS-A without measurable release. In the same way, synthetized and retained SRS-A could contribute to the trachea response in the subsequent challenge thereby combating the desensitization process.

3. Respiratory distress in conscious guinea-pigs

Ovalbumin-sensitized guinea-pigs were placed in a closed chamber exposed to a nebulized solution of ovalbumin or histamine. The concentration of the ovalbumin solution was 5 % and that of histamine 0.04 %. The method is described in detail by Olsson (1971). Latency time was determined for the onset of respiratory distress defined by a marked drop in frequency of respiration and the appearance of spasm in the diaphragm region. The exposition was stopped when these signs appeared. Maximum exposure time was 10 minutes. The protecting effect of TRB, subcutaneously administered 15 minutes before exposure to histamine or ovalbumin, was measured as a significant increase of exposure time before the appearance of respiratory distress.

## V ANTI-ANAPHYLACTIC EFFECTS OF β-ADRENOCEPTOR STIMULANTS

### 1. Inhibition of histamine release from chopped lung (I, II, III, VI, VII)

IPR, $2x10^{-8}$ M - $2x10^{-6}$ M, inhibited about 65 % of the ovalbumin-induced release of histamine (Fig. 2 A). The effect was blocked with propranolol. Variation of pre-incubation time for IPR in the range 2-30 minutes before the addition of ovalbumin did not change the effectivity of IPR (I). Assem and Schild (1971 a) found good inhibition when IPR was added together with antigen; Orange *et al*. (1971) did so too when it was added 1 minute before antigen to chopped human lung.

Within a concentration range of antigen causing dose-dependent release of histamine, IPR was found to show inhibition (I). Assem and Schild (1971 a) reported that antigen in excess impaired the inhibitory effect of IPR on the histamine release from the actively sensitized guinea-pig lung. Their findings perhaps depend on over-stimulation with antigen on the analogy of the findings of van den Brink (1973) in experiments with tracheal muscles.

The antigen-induced release of histamine was markedly reduced upon depleting calcium ions from the incubation fluid by EDTA (I). The residual histamine release could not be further reduced by IPR. However, when calcium ions were present in excess, the normal histamine release was obtained, and an inhibitory effect with IPR reappeared.

The detergent Triton X-100 is a nonselective releaser of histamine from the guinea-pig lung (Hitchcock 1973). When Triton X-100, 0.10 %, was added to the guinea-pig lung, about 20 % of tissue content of histamine was released (Fig. 2 B). However, isoprenaline, $2x10^{-7}$ M, when added 2 minutes before the detergent did not show any effect on the histamine release (Fig. 2 B). The antigen-induced

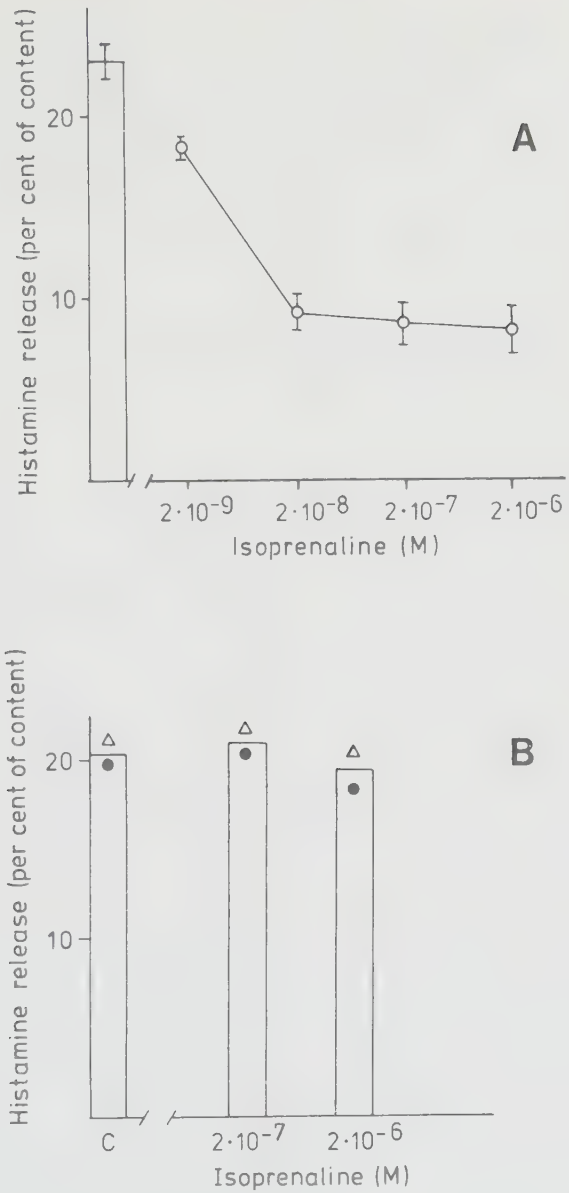


Fig 2

A. The inhibitory effect of IPR on histamine release from actively sensitized guinea-pig lung induced by ovalbumin (1 mg/ml). Each point represents the mean ± S.E.M. (n = 4).

B. The release of histamine from actively sensitized guinea-pig lung with Triton X-100, 0.10 %, alone (c) and in the presence of IPR. Bars indicate the means of two experiments.

histamine release from rat mast cells is reported to take place with retained viability of the cells (Bloom and Hägermark 1965). Johnson and Moran (1970) found that adrenaline, IPR, and noradrenaline inhibited the anaphylactic release of histamine from peritoneal rat mast cells. But none of the compounds inhibited the release induced by Triton X-100.

Obviously, the inhibitory effect of IPR is restricted to the selective and calcium-dependent release of histamine from the guinea-pig lung.

TRB counteracted the histamine release from the sensitized guinea-pig lung (II). It was about 1/20 as potent as IPR. The effect was completely blocked by β-blocking agents, which indicated the involvement of β-adrenoceptors. Ibuterol, the di-isobutyric acid ester of TRB, showed a clear selectivity for trachea relaxation in vitro (V and fig. 1). It is also more lipophilic than TRB (cf Ryrfeldt and Bodin 1975) why it might more easily penetrate into the chopped lung tissue. Ibuterol inhibited the antigen-induced histamine release (VI). The control release with ovalbumin was inhibited in a dose-dependent way. Propranolol, $10^{-7}$ M, blocked the effect. When the esterase inhibitor eserine was present in the incubation fluid, the inhibitory effect of ibuterol was blocked and that of TRB was unchanged. It is concluded that ibuterol is effective as inhibitor of the antigen-induced histamine release by β-adrenoceptor stimulation. A prerequisite for the effect seems to be hydrolyzation of the ester to TRB and isobutyric acid, analogous with the findings in the experiments with isolated cat bronchi (V) and isolated atrial and tracheal preparations from guinea-pigs (Bohmer and Raper 1976). The higher lipophily of ibuterol did not improve the efficacy on the anaphylactic histamine release in comparison with TRB.

The inhibitory effect of the compounds IPR, OPR, ITP, and TRB on histamine release was compared with their relaxing

effect on the guinea-pig trachea and their stimulating effect on the guinea-pig heart (III). IPR, OPR, and TRB inhibited the histamine release in a concentration-dependent way and to the same maximum level. The effect was blocked by propranolol. ITP, however, did not show any inhibition. The order of potency of the four agents was IPR > OPR ≈ TRB >> ITP. This pattern was more related to that found for trachea relaxation (IPR > TRB > OPR >> >> ITP) than for increase in heart rate (IPR > OPR > ITP≈ ≈ TRB) or force of contraction (IPR > OPR > ITP > TRB).

In this study (III) the following experimental factors are considered: 1) The agonists did not show α-adrenoceptor stimulation in the concentrations used. 2) The response of a given concentration of agonist was measured at the peak level of the response. 3) Indirect evidence is presented showing that the agonists are not actively taken up into adrenergic nerves. 4) The conclusion drawn from the rank order of potency cannot be disturbed by enzymatic degradation of the agonists. 5) Changes in sensitivity of tissue preparations were controlled by using IPR as a reference substance in the experiments.

The experimental conditions for the achievement of the effects of the four agonists are considered to fulfil the requirements for regarding the rank order of obtained potencies as reflecting properties at the receptor level (Furchgott 1972).

The β-adrenoceptors of the guinea-pig lung mediating inhibition of antigen-induced release of histamine are qualitatively neither the same as those mediating trachea relaxation nor those mediating heart stimulation (III), but with some relation to the former type. This sign of difference between the anti-anaphylactic β-adrenoceptors and those in tracheal smooth muscle was further substantiated by a new β-adrenoceptor stimulant, KWD 2131 (VII). This compound is structurally related to TRB and shows

the same potency on inhibition of anaphylactic histamine release as the latter compound, but the potency on trachea relaxation and heart muscle stimulation was low (0.04 x TRB). The effects were blocked by propranolol. KWD 2131 lacked α-stimulating properties in the concentrations used. The preliminary findings show that KWD 2131 is a β-adrenoceptor stimulant with especially anti-anaphylactic property. A broader pharmacological investigation of this compound is in progress.

Malta and Raper (1975) characterized the anti-anaphylactic β-adrenoceptors by six non-catechol β-stimulating agents. Unlike the present findings (III), none of these compounds (i.e. TRB and OPR) produced the same maximum inhibition of histamine release as did IPR. Moreover, they found OPR more potent than TRB, whereas the two compounds were equipotent in the present study. Malta and Raper found no clear similarities with the rank orders found in the atrial and tracheal preparations. They concluded that the β-adrenoceptors involved in regulation of the histamine release differ from those in the tracheal smooth muscles and also those in the atrial preparation. This heterogeneity was further substantiated by studies of the $K_B$ values of the cardioselective antagonist H 93/26 in atria, trachea, and lung anaphylaxis. All these $K_B$ values differed from one another. Malta and Raper came to the same conclusion as did Assem and Schild (1971 b) who compared the antagonistic effects obtained with butoxamine and practolol in anaphylactic histamine release *in vitro* with other effects *in vivo*. The latter concluded that the β-adrenoceptors in anaphylaxis differ from those responsible for heart rate increase in the cat ($\beta_1$) and vasodepression ($\beta_2$) in the dog, but are related to those mediating lipolysis in the dog.

The present knowledge of the anti-anaphylactic β-adrenoceptors of the guinea-pig lung, based on studies with β-agonists and β-antagonists, makes it difficult to range

them in the $\beta_1$ - $\beta_2$ scheme of Lands. Similar difficulties have been shown for the β-adrenoceptors of the blood vessels (Bristow et al. 1970, Wardell et al. 1974, Wasserman and Levy 1974).

In view of the present findings, the β-adrenoceptors of the organism can be considered a population of receptors where β-adrenoceptors of different cell types have a spectrum of subtle quality differences, which might be more or less easily discovered, depending on the chemical structure of the pharmacological agents used.

2. Effect of TRB on histamine release contra effect on tracheal smooth muscle

The mean $EC_{50}$ value (effective concentration for 50 % relaxation) for TRB was 21 ng/ml (III). Inhibition of histamine release from the sensitized lung tissue was obtained within the concentration ranges 4-400 ng/ml (II) and 10-1000 ng/ml (III). Thus it is possible for TRB to exert relaxation and inhibition of histamine release in the same concentrations.

To study this further, the effect of TRB on the anaphylactic contraction of the isolated guinea-pig trachea was investigated (IV). The preparations were challenged by a low dose of ovalbumin causing suboptimum contractions (25-35 % of maximum). By intermittent washing of the preparation during 1 hour, it was possible to reproduce the same amount of contraction. Four contractions were obtained on the same preparation. Higher concentrations of ovalbumin or shorter time between successive challenges caused marked desensitization, but the response to a standard dose of histamine was unchanged.

Trachea preparations from sensitized guinea-pigs were treated with low concentrations of TRB, 2-4 ng/ml, 5 min before challenge with standard concentration of antigen.

The development of anaphylactic tone was markedly reduced (IV). The same TRB doses, however, were less effective when added at the peak effect of antigen. As this improved 'prophylactic' effectiveness of TRB was found exclusively when tone was elicited by antigen and was missing when the same amount of tone was induced by the three naturally occurring mediators in the lung, histamine, acetylcholine, and $PGF_{2\alpha}$, it is considered an indication that TRB is effective by inhibiting the antigen-induced release of muscle-contracting agents in the sensitized trachea.

3. Effect of TRB on respiratory distress in sensitized guinea-pigs

TRB protects the intact guinea-pig from respiratory distress induced by inhaled histamine (V). With the same aerosol technique, sensitized guinea-pigs were exposed to ovalbumin and histamine.

Results show (Fig. 3) that ovalbumin and histamine caused respiratory distress within a 1.5-min stay in the aerosol chamber. TRB, 0.2 - 1.0 mg/kg, subcutaneously administered 15 min before exposure to the aerosol increased the latency time. There was no significant difference in the protective effect of TRB between the two ways of inducing respiratory distress.

It is concluded from the *in vivo* experiments that TRB protects the animals from anaphylactic bronchoconstriction in the same concentrations as are needed for protection against respiratory distress induced by one of the mediators in anaphylaxis, histamine. Thus the greater prophylactic efficacy of TRB, found in trachea anaphylaxis (IV), was not present *in vivo*. This is perhaps because the provocation dose of ovalbumin *in vivo* had to be kept high in order to get a well-defined starting point for the respiratory distress. Thereby the anti-anaphylactic effect of TRB was insufficient to protect the animal.

Another reason may be that the high concentration of ovalbumin affecting irritant receptors in the bronchial tree which in turn induced bronchoconstriction reflexogenously.

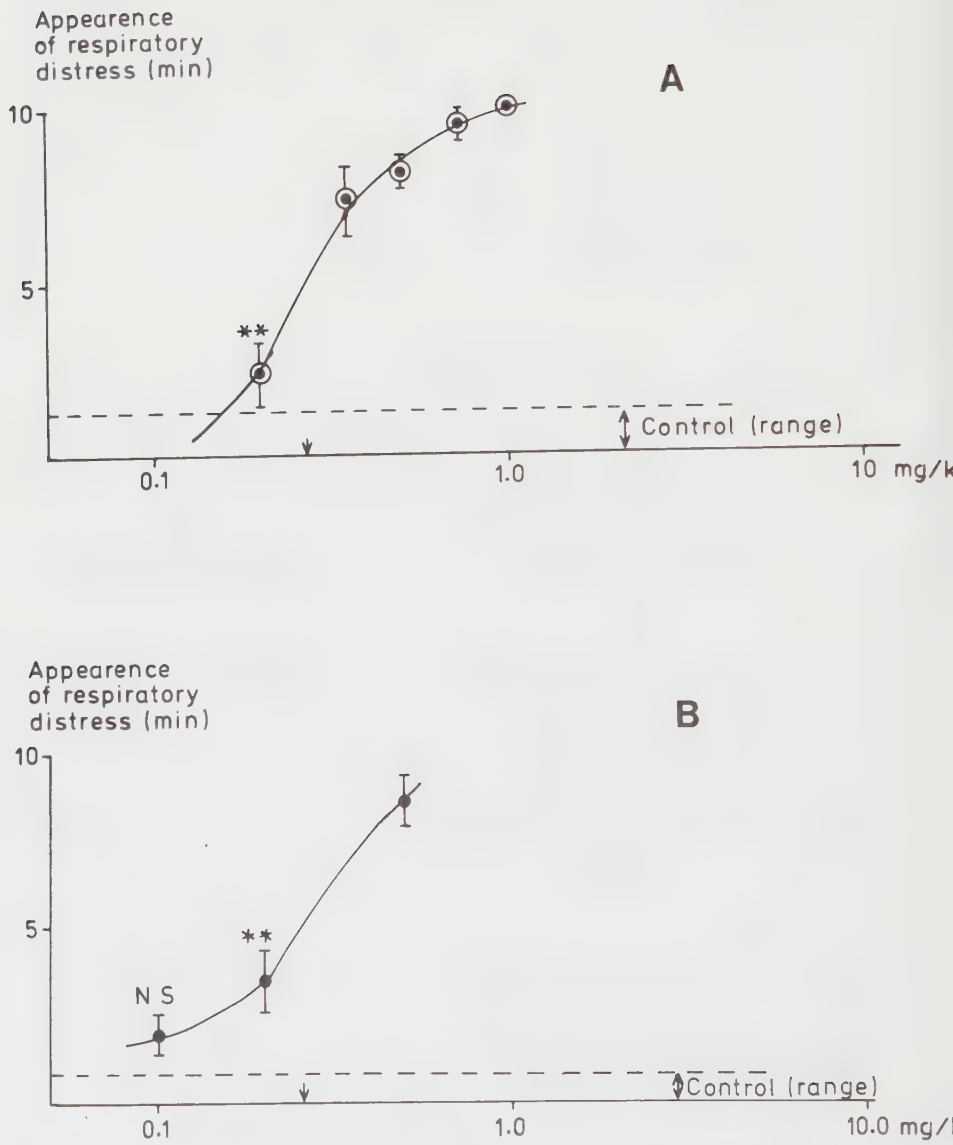


Fig 3

The protective effect of TRB against the appearance of respiratory distress in conscious guinea-pigs. TRB was administered subcutaneously 15 min before challenge to aerosolized ovalbumin (A) or histamine (B). Each point represents the mean ± S.E.M. of 5 animals.

## VI PRINCIPAL FINDINGS

The principal findings of the present study can be summarized thus:

1/ The inhibitory effect of IPR on anaphylactic histamine release from guinea-pig lung is present within a concentration range of antigen, where the amount of histamine release is related to the antigen dose. The time of preincubation with IPR is not critical for the degree of inhibition. Moreover, the IPR effect is found to be restricted to the calcium-dependent and non-cytotoxic release of histamine.

2/ The β-adrenoceptors of the guinea-pig lung mediating inhibition of antigen-induced histamine release are characterized by four β-adrenoceptor stimulants with three different profiles of action on tracheal and heart muscle. The actual receptors are not of the same quality as those in the heart or those in the tracheal smooth muscle, but are related to the latter.

The preliminary description of a new β-adrenoceptor stimulant, KWD 2131, with preferably anti-anaphylactic property and low potency on tracheal smooth muscle and heart, suggests that the anti-anaphylactic β-adrenoceptors of the guinea-pig lung differ from those in the tracheal smooth muscle and those in the heart muscle.

3/ The bronchoselective β-adrenoceptor stimulant TRB inhibits the anaphylactic histamine release in guinea-pig lung tissue. Effective concentrations are about the same as needed for relaxation of the isolated trachea contracted by pilocarpine. There is evidence in experiments with trachea preparations from sensitized guinea-pigs that TRB, when added before antigen, inhibits the contraction to some extent via the anti-anaphylactic mechanism. TRB protects the conscious guinea-pig from anaphylactic respiratory distress in the same concen-

tration range as it protects against histamine-induced respiratory distress.

4/ The diisobutyric acid ester of TRB, ibuterol, is characterized pharmacologically. The profile of action of ibuterol is that of a bronchoselective β-adrenoceptor stimulating agent. It is effective after having undergone hydrolyzation to TRB and isobutyric acid on the anaphylactic histamine release from the guinea-pig lung. Ibuterol can be considered a predrug for the active agent TRB.

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